2019-Pos Board B38
AFM/TIRFM Study of Individual Fibrin Fibers
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Colocalized imaging of individual fibrin fibers by combined atomic force microscopy (AFM) and total internal reflection fluorescence microscopy (TIRFM) was used to gain insights into the structure and the effects of the degree of hydration. In AFM images obtained under buffer conditions, fibrin fibers appear flattened, with heights one order of magnitude smaller (tens of nm) than diameters (hundreds of nm), with thicker fibers more flattened. All fibers present thickness variations along their length, of up to 3 nm in diameter per 10 nm length of fiber. Branching of fibers, at angles between 0-900 was also observed. High resolution imaging reveals the periodic structure of fibers, with a periodicity of 23 nm corresponding to half-staggering of protofibrils. Exchange of the physiological buffer with water increases the cross-sectional area of individual fibers to more than double. In dehydration and rehydration experiments, identical fibers show large variations in cross-sectional area. Upon drying, cross sections decrease by up to more than 80% while upon rehydration the same cross sections increase by a factor of up to three, relative to the dry value. TIRFM imaging of the same fibers showed that fibers fluorescence correlated with the topographical features imaged by AFM, suggesting that labeled protein is uniformly distributed throughout the fibers. Single-molecule TIRFM imaging of fibrinogen molecules, basic constituents of fibers, allows for a molecular calibration that determines the number of molecules in individual fibers; correlated with AFM topographical data informs to the degree of hydration of the fibers - a major question in the fibrin fiber structure.

2020-Pos Board B39
Mass-Per-Unit-Length Determination by Stem
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Mass-per-unit-length is key to defining filament symmetry. STEM measures mass and length simultaneously on freeze-dried, unstained specimens. The accuracy is usually better than 5% for mass and 0.5% for length. Spatial resolution is typically 3nm at an electron dose of 10el/Å^2, so heterogeneity can be measured along the traced length. Also, the projected mass profile determined directly from the image can be compared with simulated models or transformed to a radial density profile. Tobacco mosaic virus (TMV) is included in all specimens as an internal control for structural preservation and freedom from salt artifacts. Application to prions, amyloids and filamentous viruses will be described.
The Brookhaven STEM operates at 40 keV with a probe size of 0.3nm obtained by focusing a beam from a cold field emission source. The specimen is maintained at −160°C to reduce mass loss. Specimens are fast frozen, freeze dried overnight and transferred under vacuum to the microscope. Quantum efficiency detectors measure every electron passing through the specimen as: unscattered (bright field), scattered at small angles (SA) or scattered at large angles (LA, dark field). The LA signal is used for mass measurements since it is free of coherent scattering effects (defocus phase contrast, diffraction contrast on crystals). Off-line analysis masks particles to determine background, finds filament segments and measures signal minus background, converting to mass-per-unit-length. All measurements are stored in a database with statistical analysis. http://www.bnl.gov/biology/stem/

2021-Pos Board B40
Aggregation and Fibril Formation in Oligo-Glu. Use of Isotope Edited Vibrational Spectra to Assign Structure
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Aggregation and fibrilization have been determined to be ubiquitous properties of protein and peptide systems placed under various forms of stress. Most such systems have secondary structures with high beta sheet content, but this can vary greatly in detail. While the most fundamental variance is the difference in parallel and anti-parallel interstrand alignment leading to cross-strand H-bond formation, the registration of identical strands, their twisting, and for fibrils, the relative orientation of stacked sheets is also important in understanding these basic protein interaction processes that have important biomedical consequences. We have chosen to model the process using glutamic acid peptides due to some of their unique properties. At low pH, polyGlu forms fibrillar structures with unique IR signatures in the amide I region, which have been assigned as arising from bifurcated H-bonding (β2 form). We have shown the same patterns can develop in oligo-Glu (Glu10) and various labeled mutants (using 13C-Val to incorporate labels on the amide C=O), and that with isotope labeling we can assign the structures to have antiparallel sheet formation and most likely being out of register by one residue. IR and VCD data are shown to be consistent with computational models based on idealized sheet structures. Addition of the sidechain effects on the spectral response is being modeled as well as the effects of stacking in the fibrillar structure.

2022-Pos Board B41
Characterizing the Role of Palindromic Strand Exchange in Alpha Crystallin Oligomerization
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The small heat-shock protein (sHSP) alpha-crystallin is a major component of the eye lens where it performs critical functions in maintaining lens transparency. Alpha-crystallin acts as a molecular chaperone to prevent stress-damaged and aging proteins from forming light-scattering aggregates. Further, a high concentration of alpha-crystallin contributes to a large and uniform refractive index across the lens tissue. Despite being expressed in such high concentrations, alpha-crystallin does not form aggregates itself, a property facilitated by the assembly of alpha-crystallin into polydisperse, dynamic oligomers that frustrate aggregation and crystallization. Structural studies suggest that one mechanism underlying this polydisperity may be the ability of a palindromic sequence centered on the sHSP IXI motif in the alphaB-crystallin isoform c-terminus to bind bidirectionally to other monomers. Strand exchanges formed in this manner would result in varied oligomeric structures while maintaining near identical residue interactions. We are using Förster resonance energy transfer (FRET) to measure the ability of the palindromic sequence to bind in differential orientations in solution and to determine any intrinsic directional bias. These data will help determine whether bidirectional strand exchange is important for αB-crystallin function by assaying how strand binding influences chaperone activity. Our work will build upon the proposed mechanism of alpha-crystallin polydisperity and clarify the importance of bidirectional strand exchange for alpha-crystallin function.

2023-Pos Board B42
Folding and Unfolding of pH Sensitive Peptides: The Role of Interfaces
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GALA is a 30 amino acid synthetic peptide consisting of a Glu-Ala-Leu-Ala repeat, known to undergo a reversible structural transition from an unordered to an α-helical structure when changing the pH from 7 to 5. In its helical state GALA is amphiphilic and can insert into and permeabilize membranes. This effect has generated much interest because of potential applications for pH triggered targeted drug delivery. GALA also serves as a well-defined model system to understand cell penetration mechanisms and protein folding triggered by external stimuli. The structural reconfiguration of GALA in solution has been studied extensively. However, cell penetration is an interfacial effect and occurs at the membrane surface. Owing to experimental challenges in determining peptide confirmation at an interface, GALAs interaction with surfaces is still unknown. A key question is: What is the secondary structure of GALA specifically at interfaces such as the air-water or water-lipid interfaces? In other words: how does the presence of an interface affect the intricate balance of forces governing folding and unfolding of GALAs Glu-Ala-Leu-Ala motive? We have used sum frequency generation vibrational spectroscopy to probe the structural response of GALA at the air-water interface. SFG is highly interface specific and only probes peptides directly, i.e. within a few Angstrom, at the water-air or water-lipid surface. The data reveal that a large fraction of the GALA population at the water-air interface remains helical above pH 5, while a complete reconfiguration into the unordered state was observed in solution by transmission infrared and circular dichroism spectroscopy. We attribute this effect to the stabilizing interactions of hydrophobic leucine side chains with air. Molecular dynamics simulations support the view that the surface plays a key role in the balance of structure-building forces at the interface.

2024-Pos Board B43
Fluorescence Anisotropy in a Protein: DNA System Undergoing Inducible Assembly
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Fluorescence anisotropy may be used as an indicator of homo-FRET making it an attractive reported of oligomer formation and aggregation. A model system was created consisting of a monomeric teal fluorescent protein peptide nucleic acid (mTFP-PNA) fusion. The mTFP-PNA monomer units produced by expressed protein ligation (EPL). An mTFP with a C-terminal thioester group was expressed with a modified intein and subsequently conjugated to a PNA with an N-terminal thiol group. This generates a native peptide bond at the ligation site. Addition of a complementary template DNA to